

Montana 2007 Avian Influenza Surveillance Project Report

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EXECUTIVE SUMMARY

Avian influenza (AI) is a type-A influenza virus enzootic in wild bird populations for which waterfowl and shorebirds, in particular, have been identified as reservoirs in nature. The strain of AI currently causing global concern is the highly pathogenic H5N1 Asian strain (HP-H5N1). The emergence and recent spread of HP-H5N1 in Asia, the Middle East, Europe, and Africa has elevated apprehension about potential expansion of HP-H5N1 to North America. Such an event could have negative affects on the poultry industry, humans, and wild bird populations (World Health Organization 2007b). The U.S. Department of Agriculture and the U.S. Fish and Wildlife Service initiated a nationwide avian influenza surveillance project for the early detection of HP-H5N1 in 2006, which was continued in 2007. Montana was considered a top priority state because the Pacific and Central Flyways divide the state and it borders Canada.

The objectives of this project were to employ multiple sampling strategies to maximize the chance of detecting HP-H5N1, including sampling live and hunter-harvested waterfowl throughout fall migration, collecting environmental samples from areas of high waterfowl concentration, and collecting samples from wild bird mortality/morbidity events. To achieve the 2007 objectives, personnel from Montana Fish, Wildlife and Parks and USDA-APHIS Wildlife Services collected 1502 swab samples from live and hunter-harvested birds, 649 environmental samples, and 59 mortality/morbidity samples. Six weekly prospective mortality transects (n=103) were also conducted on lakes and wetlands throughout the state to systematically record the presence of target bird populations and mortality events.

AI virus in low pathogenic form was detected in Montana samples as expected, while HP-H5N1 was not found during 2007 in Montana or elsewhere in North America. One male hatch-year Mallard tested H5N1 positive via real-time reverse transcription-polymerase chain reaction and virus isolation, but was classified as low pathogenic using target amino acid sequence analysis. This was the only bird determined to have H5 and N1 linked in the same strain during the two years of surveillance in Montana. However, the low pathogenic classification means the HP-H5N1 Asian strain of concern was not detected.

The national 2008 AI surveillance is underway. Mortality/morbidity transects and environmental sampling began in July and sampling of live birds in Montana began in August. Opportunistic mortality/morbidity samples are collected throughout the year.

INTRODUCTION

Influenza is a respiratory disease that has infected animals and humans throughout recorded history (Webster et al. 2006). Avian influenza (AI) is a type-A influenza virus enzootic in wild populations of more than 100 bird species that rarely is expressed clinically (AHAW Panel 2006). Waterfowl and shorebirds in particular have been identified as reservoirs for the virus in nature (Olsen et al. 2006, Krauss et al. 2007).

Influenza viruses are classified by two proteins expressed on the surface of the virus, hemagglutinin and neuraminidase. There are currently 16 subtypes of hemagglutinin (H1-H16) and 9 subtypes of neuraminidase (N1-N9) that have been detected in bird populations worldwide (Munster et al. 2005). Pathogenicity, the ability to cause disease, in AI viruses may be distinguished as low pathogenic (LPAI) and highly pathogenic (HPAI) based on genetic features of the virus and the severity of the illness they cause in infected poultry (Centers for Disease Control and Prevention 2007). Most AI strains are classified as LPAI because they typically cause little or no clinical sign of disease (Munster et al. 2005, Brown et al. 2006, Olsen et al. 2006). While influenza viruses are normally highly species-specific (World Health Organization 2007b), HPAI causes severe illness and death in poultry, and can also cause disease in humans and some mammals (Olsen et al. 2006, Webster et al. 2006). LPAI viruses containing hemagglutinin of subtypes H5 and H7 may become highly pathogenic after introduction to poultry (Munster et al. 2005).

The strain of avian influenza currently causing global concern is the highly pathogenic H5N1 Asian strain, hereafter referred to as “HP-H5N1”. The emergence and recent spread of HP-H5N1 in Asia, the Middle East, Europe, and Africa has resulted in impacts to the poultry industry and presents an important threat to human health. Concern has elevated about the potential expansion of HP-H5N1 to North America and possible negative effects to the poultry industry, danger to humans on a large scale through mutation or recombination, and illness and mortality in wild bird populations (World Health Organization 2007b). While HP-H5N1 infections in humans are rare, they can result in severe illness and death. The current death rate of known human infections is approximately 60% (World Health Organization 2007a). Though H7 infection in humans is also extremely rare, conjunctivitis can occur among people who have direct contact with infected birds (Webster et al. 2006).

The role of wild birds in the movement and transmission of HP-H5N1 is poorly understood and strongly contested (Krauss et al. 2007, Peterson et al. 2007, van Gils et al. 2007). Circumstantial evidence suggests wild waterfowl may introduce AI viruses in the low pathogenic form to poultry flocks and some species of migratory waterfowl may carry HP-H5N1 to new geographical areas during migration (World Health Organization 2007b). The pathways by which HP-H5N1 has and will spread between countries have been debated extensively. Surveillance of wild ducks in the Northern Hemisphere showed a high prevalence of LPAI virus in primarily juvenile birds (~60%) in early fall before southbound migration, which then fell sharply. Waterfowl and shorebird influenza genetic data from the Americas indicate interplay between these host species. Molting, migration stopovers, and wintering grounds allow birds to exist in high densities and provide opportunities for the transmission of LPAI viruses between wild and captive birds, and between species (Olsen et al. 2006). Research on wild bird migration in combination with movements in the poultry and wild bird trade showed that most HP-H5N1 introductions to Asia were likely through poultry, most

spread to Europe was likely through migratory birds, and movement in Africa was likely caused by both poultry and migrating wild birds. While some expect HP-H5N1 to enter North America from the north through the migration of wild birds from eastern Siberia, surveillance in Alaska shows very low AI infection rates (0.06%), which suggests that frequency of intercontinental virus transfer is low (Winker et al. 2007). Given the unregulated importation of poultry in Mexico and Brazil, Kilpatrick et al. (2007) predict HP-H5N1 may be introduced to the Western Hemisphere through infected poultry and to mainland United States by subsequent movement of migrating birds from southern neighboring countries. Local North American bird populations may amplify the disease and act as wild sentinel birds from which the arrival of HP-H5N1 may be detected (Brown et al. 2006).

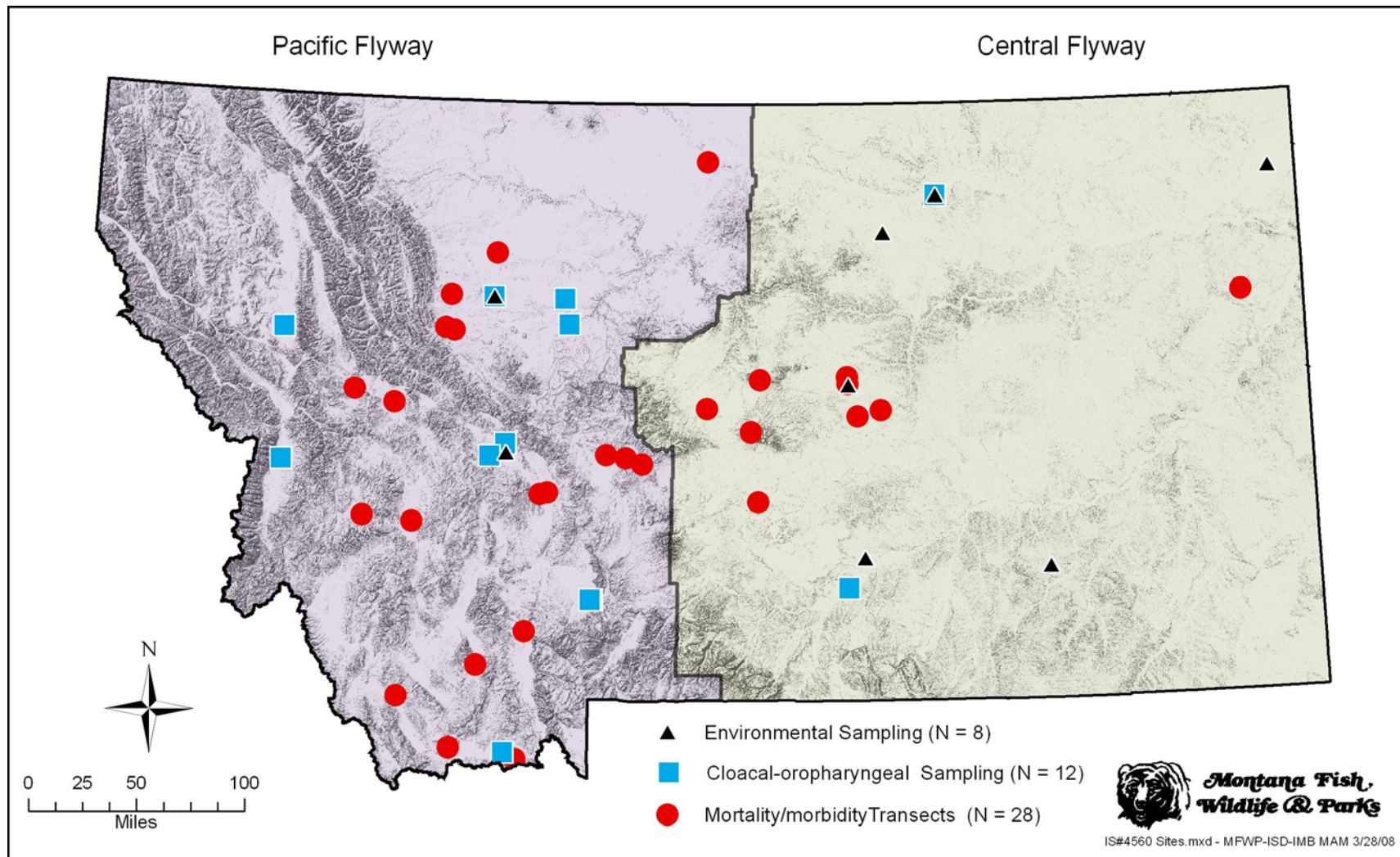
The U.S. Department of Agriculture (USDA) and the U.S. Fish and Wildlife Service (USFWS) initiated a nationwide avian influenza surveillance project for the early detection of HP-H5N1 in 2006, which was continued in 2007. The surveillance included all four flyways, all states, and tribal lands in the United States. The Pacific Flyway was considered a top priority to sample waterfowl and shorebirds potentially en route from Russia during the fall migration. Montana was considered a top priority state because the Pacific and Central Flyways divide the state and it borders Canada. Montana Fish, Wildlife and Parks (MFWP) and USDA-APHIS Wildlife Services (WS) conducted the 2007 Montana AI surveillance project sample collection and the Montana Department of Livestock (MDoL) and the USGS National Wildlife Health Center (NWHC) laboratories tested the samples. The Department of Public Health and Human Services and the Tribal Nations were also collaborators in the 2007 nation-wide effort. The objectives of the Montana project were to sample live and hunter-harvested waterfowl for the potential early detection of HP-H5N1 throughout fall migration, collect environmental samples from areas of high waterfowl concentration, and collect samples from wild bird mortality/morbidity events in the state of Montana as part of the national interagency surveillance.

STUDY AREA

Montana is the fourth largest of the 50 states with an area of more than 93 million acres. Elevations range from 1,900 feet along the Missouri River to the highest point, Granite Peak in south-central Montana, at 12,850 feet. Topography is highly varied across the state ranging from the coniferous forests of the Rocky Mountains and associated foothills in the western third to expansive prairies of the Great Plains in the eastern two-thirds of the state (Figure 1). Land ownership is comprised of over 60 million acres of private and tribal lands (65%) and nearly 28 million acres (30%) of federal lands, while state owned lands account for over 5 million acres (5%) (Montana Fish and Game Department 1971). Ecotypes vary and include montane forests, intermountain and foothill grasslands, shrub grasslands, and plains grasslands and forests, each of which includes aquatic and riparian zones.

The Pacific and Central Flyways divide Montana; the Pacific Flyway contains Hill, Chouteau, Cascade, Meagher, and Park counties and all counties west, while the Central Flyway includes Blaine, Fergus, Judith Basin, Wheatland, Sweet Grass, Stillwater, and Carter counties and all counties east. Of the 413 bird species documented in the state, 268 breed and 145 use stopover sites in Montana during seasonal migrations or occasionally occur in the state.

Figure 1. The Pacific and Central Flyways in Montana, and sampling sites for the 2007 Montana AI Surveillance Project.



METHODS

Sample Design

The 2006 Montana AI surveillance sampling strategy was a step-down approach from the U.S. Interagency Strategic Plan (Interagency Asian HPAI Early Detection Working Group 2006) and the Pacific and Central Flyway plans (Pacific Flyway Council 2006, Central Flyway Council 2006). The Montana Sampling Plan Supplement for 2007 outlined changes to the 2006 sample design to implement modifications based on the most current research on sampling protocols and target species (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). The above plans suggested that ≥ 200 samples would be required to detect one positive HP-H5N1 sample in a defined bird population of >1000 individuals with a 95% confidence interval at a disease prevalence of $\leq 1.5\%$. The national and flyway plans placed emphasis on particular species in specific areas and multiple sampling strategies were employed to maximize the chance of detecting HP-H5N1. Investigating disease events in dead or dying birds was considered one of the best opportunities to detect the potential introduction of HP-H5N1 into Montana by wild migratory birds (Wobeser 2006). Wild live and hunter-harvested bird surveillance enabled the selection of species that represented the highest risk of exposure to HP-H5N1, which included birds that migrate directly between Asia and North America (primary species) and/or mix in Alaska staging areas with species that could bring HP-H5N1 from Asia (secondary species). Environmental sampling allowed for the analysis of fecal material from waterfowl habitats because viable AI virus can be detected in feces for a period of time in cool temperatures (Interagency Asian HPAI Early Detection Working Group 2006). Surveillance efforts were accomplished through the extensive cooperation of MFWP, WS, USFWS, and city and/or county managers where the urban trapping was conducted.

Cloacal and Oropharyngeal Sampling

Cloacal and oropharyngeal sample design assumptions included 1) the populations of birds to be sampled were homogeneous and accessible, 2) HP-H5N1 was uniformly distributed across bird populations, and 3) representative sampling would be random and unbiased. Because these assumptions could not be met for wild migratory waterfowl, sampling was increased in an attempt to account for biases and sample sizes were extrapolated across large landscapes for multi-state and flyway sampling efforts (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006). Cloacal and oropharyngeal sampling targeted specific species spatially distributed across Montana and temporally distributed from August through December. Species of primary concern for the 2007 AI live and hunter-harvested bird surveillance in Montana included tundra swan (TUSW), lesser snow goose (LSGO), northern pintail (NOPI), and Ross's goose (ROGO). These species move between Asia and North America and could contact the Asian HP-H5N1 directly (Alaska Interagency HPAI Bird Surveillance Working Group 2006). Secondary and wild sentinel species included mallard (MALL), American wigeon (AMWI), gadwall (GADW), and northern shoveler (NSHO). Additional priority species were blue-winged teal (BWTE), common goldeneye (COGO), canvasback (CANV), green-winged teal (AGWT), redhead (REDH), and wood duck (WODU). High numbers of these species migrate through the state and provide opportunity for sampling through banding operations, waterfowl hunting, and urban trapping (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006). Hybrid semi-domestic geese and ducks served as sentinel species and were sampled at urban ponds.

Field

Changes in the 2007 AI surveillance design from 2006 included screening all swab samples individually rather than pooling samples. As a result, the target number of swab samples was reduced to adjust for the cost of initial screening. Sampling criteria for 2007 stated that MFWP and WS should each collect 750 cloacal-orpharyngeal samples from birds identified as species of concern for a total of 1500 samples statewide. Based on recent research examining HP-H5N1 shedding, the 2007 sampling protocol also included the addition of an oropharyngeal swab placed in the same vial with a cloacal swab to amplify the sample (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). MFWP and WS collaborated in their sampling efforts to achieve the 2007 objectives. Three strategies were employed for cloacal and oropharyngeal sampling: coordinating with USFWS National Wildlife Refuge waterfowl banding operations, sampling hunter-harvested waterfowl at National Wildlife Refuges and on state-owned lands, and trapping wild and semi-domestic waterfowl on urban ponds across the state.

Live bird AI sampling performed in conjunction with National Wildlife Refuge banding was conducted at Benton Lake and Bison Range/Ninepipes during September using methods approved by the U.S. Fish and Wildlife Service and Canadian Wildlife Service (1977). Swim-in traps were employed at three locations at Bison Range/Ninepipes while net-launchers were used at three sites at Benton Lake. Trapping efforts were rotated between sites at both refuges. Waterfowl were first banded by USFWS and tribal biologists, cloacal and oropharyngeal samples were then taken by WS and MFWP AI personnel, and the birds were released.

Urban wild and semi-domestic bird sampling began in mid-August and ran throughout the sampling period except during September when refuge banding operations were underway. AI personnel used swim-in traps at six urban ponds across the state to collect cloacal and oropharyngeal samples. Because swim-in traps required a flat surface covered by ≤ 1.5 feet of water, traps were set in water only at Bancroft Pond in Missoula and Gibson Pond in Great Falls. Swim-in traps modified for use on land were utilized at Lewis and Clark Fairgrounds Pond in Helena, MSU Pond in Bozeman, Overland Pond in Billings, and Washoe Pond in Anaconda. Trapping at Sylvan Pond in Bozeman was conducted using land box traps. Permission to trap was granted by city and/or county managers, while MFWP Information and Education personnel and city managers worked together to notify the public of the trapping activities.

Hunter-harvested waterfowl sampling began in late September and ran concurrently with urban trapping through early December. Hunter-harvested waterfowl were sampled at Benton Lake, Bison Range/Ninepipes, Bowdoin, Lee Metcalf, and Red Rocks Lakes National Wildlife Refuges, Freezeout Lake, Lake Helena, and a site in Howard Valley in southeast Montana. Hunter participation was voluntary and information about AI and the surveillance was distributed to hunters onsite and at MFWP offices. Sampling concluded when hunting diminished as lakes froze.

The date, collector, county and site, location in WGS 84 decimal degrees, as well as the three most abundant species at each site were recorded on USDA datasheets for all cloacal and oropharyngeal sampling. Species, sex, age, condition, and band number when present for each bird sampled were also recorded. Species, sex, and approximate age were identified via plumage

(Carney 1992). Cloacal and oropharyngeal samples were taken from each live and hunter-harvested bird by gently swabbing the cloacal and oropharyngeal linings with sterile Dacron[®] swabs to obtain epithelial cells. The cloacal and oropharyngeal swabs were then placed in the same glass vial containing chilled brain-heart infusion broth for preservation. A pre-printed barcode with a sample identification number was placed on the vial, corresponding datasheet, and lab submission form to track samples from each bird. Samples were shipped overnight to the National Animal Health Laboratory Network laboratory at the MDoL Veterinary Diagnostic Laboratory in Bozeman in Styrofoam[®]-lined boxes with cold packs within 24 hours of sample collection. A sample batch referral number, the submitter, and number of samples in each shipment were recorded on the datasheet and corresponding lab submission form. Lab submission forms were sent to the MDoL lab with the samples. Datasheets corresponding to samples credited to MFWP were sent from the field to the MFWP AI Coordinator while datasheets corresponding to samples credited to WS were sent to the Montana WS wildlife disease biologist. All datasheets were then immediately faxed to the WS national database manager. An additional MFWP datasheet was used during hunter-harvest sampling to record the hunter's name, Montana license number (ALS#), contact information, bird species, and the sample barcode number to connect hunters with the birds sampled.

Lab

The MDoL lab tested each cloacal-oropharyngeal sample by real-time reverse transcription-polymerase chain reaction (rRT-PCR). All samples were initially screened individually with a matrix gene primer/probe set designed to detect all influenza-A viruses. Samples testing positive were further analyzed to identify H5 and H7 subtypes. Samples that screened positive or suspect for H5 or H7 were then sent to the National Veterinary Services Laboratory (NVSL) in Ames, Iowa. NVSL performed confirmatory testing for H5 and H7 subtypes using rRT-PCR and a standard rRT-PCR for N1. Virus isolation (VI) tests were also performed by NVSL on all samples to be confirmed to isolate AI viruses and determine whether or not H5 and N1 were linked in the same viral strain. All samples that produced positive results using VI were then tested for pathogenicity using chicken inoculation studies and/or, if enough RNA was present in the clinical sample, a target amino acid sequence analysis was performed to determine virulence potential of the virus (U.S. Department of the Interior and Wildlife Service 2006).

Sampling Effort

Cloacal and oropharyngeal sampling was performed in conjunction with refuge banding operations 9/05 – 9/26, hunter-harvested waterfowl sampling was conducted 9/22 – 11/20, which ended as fall migration subsided, and urban wild bird sampling was conducted 8/16 – 12/05. A total of 67 sampling days were comprised of 8 sample days from refuge banding operations and 15 sample days from urban pond sampling for a total of 23 days of live bird sampling. Hunter-harvest sampling was conducted on 44 sample days. Sampling effort resulted in overall means of 3.9 days/site and 22.4 samples/sample day at 17 sites across all swab sampling methods (Table 1). A total of 1502 cloacal-oropharyngeal samples were collected; banding operations yielded 261 samples (17%) and urban trapping efforts produced 191 samples (13%) for a total of 452 live bird samples (30%). Hunter-harvested samples totaled 1050 (70%; Table 2). Hunter-harvest sampling at Freezeout Lake yielded over one-third of the total swab samples collected (n=621, 41.3%). Banding operations produced the highest mean number of samples/sampling day (32.6) while urban trapping yielded the least mean number of samples/sampling day (12.7;

Table 1). Though Benton Lake banding operations produced the highest mean of 36.6 samples/sampling day, the most productive site was Freezeout Lake. The least productive sampling sites were Howard Valley and Washoe Pond (Table 2).

Table 1. 2007 Montana AI Surveillance Project swab sampling effort according to method.

	Sampling Method			Total
	Banding	Urban	Hunter-harvest	
Number of sites	2	7	8	17
Total samples	261	191	1050	1502
Percentage of total samples	17	13	70	100
Total sample days	8	15	44	67
Mean sample days/number of sites	4.0	2.1	5.5	3.9
Mean samples/sample day	32.6	12.7	22.9	22.4

Table 2. Number of sample days, and number and percentage of samples per site across cloacal and oropharyngeal sampling methods during the 2007 Montana AI Surveillance Project.

Method	Site	Sample days	Total number of samples	Percentage samples per method
Banding (live bird)	Benton Lake	6	220	84.3
	Bison Range	2	41	15.7
Total		8	261	100
Urban (live bird)	Bancroft Pond	3	0	0.0
	Gibson Pond	3	63	33.0
	MSU Pond	3	39	20.4
	Lewis & Clark Pond	2	36	18.9
	Overland Pond	2	27	14.1
	Sylvan Pond	1	25	13.1
	Washoe Pond	1	1	0.5
Total		15	191	100
Total live bird		23	452	100
Hunter-harvest (dead bird)	Freezeout Lake	25	621	59.1
	Red Rocks Lakes	3	117	11.2
	Bowdoin	4	78	7.4
	Lake Helena	4	78	7.4
	Bison Range	1	62	5.9
	Benton Lake	2	47	4.5
	Lee Metcalf	3	42	4.0
	Howard Valley	2	5	0.5
Total		44	1050	100
Sampling Total		67	1502	100

The highest proportion of samples was collected in the northeastern section of the Montana Pacific Flyway at Freezeout Lake and Benton Lake. Sampling was distributed fairly evenly across the rest of the Pacific Flyway both spatially and temporally. Cloacal and oropharyngeal sampling occurred at two sites in the Central Flyway, mostly at Bowdoin. Sampling peaked during the opening weekend of waterfowl hunting statewide (Figure 2).

The 2007 Montana Sampling Plan called for cloacal-oropharyngeal samples from 100 tundra swans, 150 lesser snow geese, and 300 northern pintails (150 from banding operations and 150 from hunter-harvest sampling) as primary species of concern, whereas the majority of secondary species samples were to come from mallards (n=760). The Montana AI team collected 93 tundra swan, 115 lesser snow goose, and 47 northern pintail samples from available birds. Ross's goose was added as a primary species for the 2007 sampling and 24 samples were collected. Primary species comprised 18.6% of the total samples collected. The 538 mallard samples collected were approximately one third of all cloacal-oropharyngeal samples collected, a significant decrease from 2006. The other secondary species of concern, gadwall (n=138), northern shoveler (n=71) and American wigeon (n=139), comprised 23.2% of the total cloacal-oropharyngeal samples, while the rest of the species sampled combined yielded 27.2% of the total samples (Table 3).

Figure 2. Temporal distribution of the 2007 Montana AI cloacal and oropharyngeal sampling; sites with <25 total samples were excluded. Scale bar numbers are the maximum number of samples collected during a two-week sample period. National Wildlife Refuge is referred to as “NWR”.

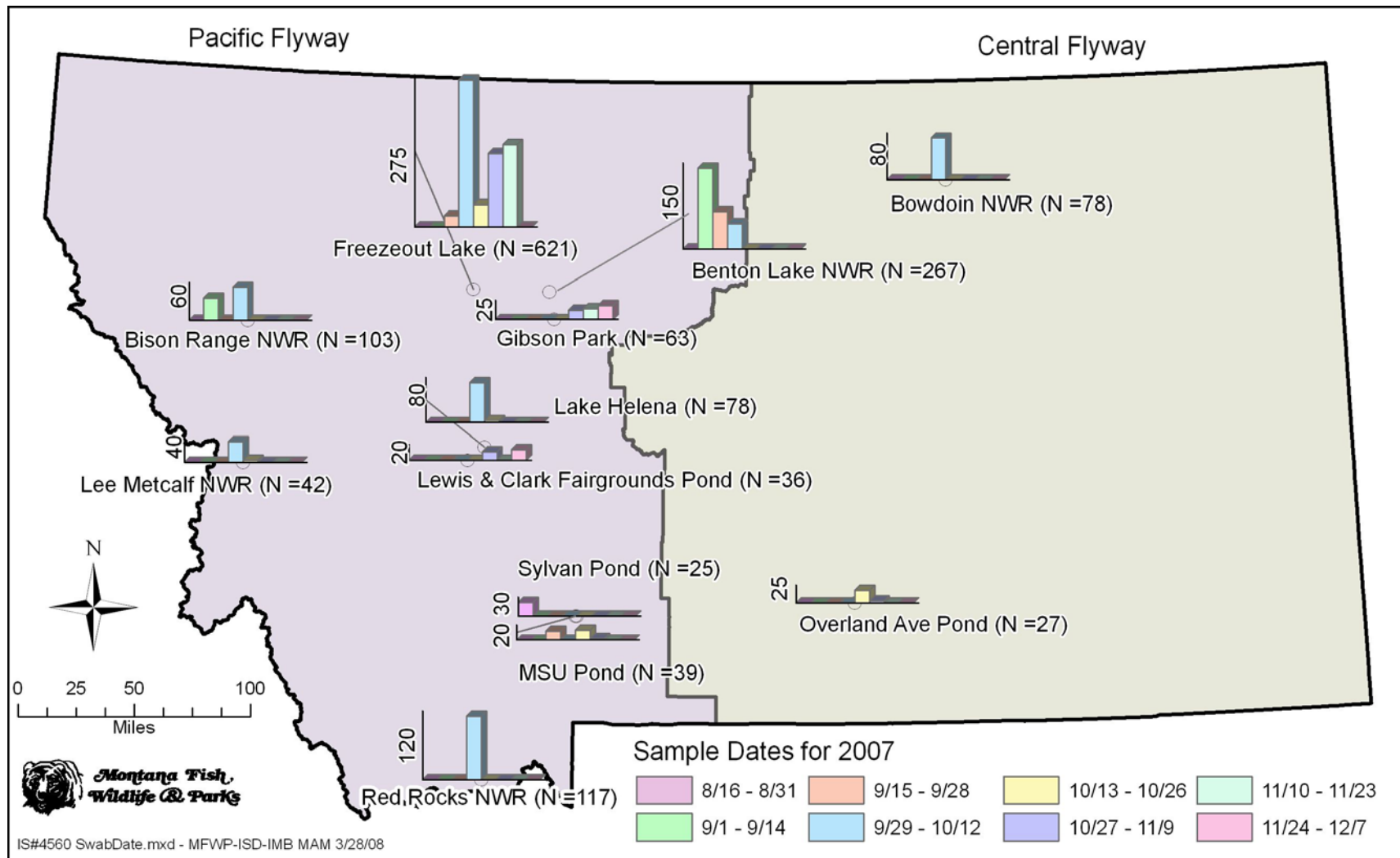


Table 3. Number of the 2007 Montana AI cloacal-orpharyngeal samples according to species and method, and percentage of total samples according to species.

Species	Banding	Urban	Hunter-harvest	Total	Percentage of total samples
Mallard	239	149	150	538	35.8
American Wigeon	3	0	136	139	9.2
Gadwall	3	0	135	138	9.2
Lesser Snow Goose	0	0	115	115	7.7
Tundra Swan	0	0	93	93	6.2
Blue-winged Teal	0	0	79	79	5.3
Northern Shoveler	0	0	71	71	4.7
Green-winged Teal	1	0	66	67	4.5
Canvasback	0	0	48	48	3.2
Northern Pintail	11	0	36	47	3.1
Hybrid Goose	0	31	0	31	2.1
Ross's Goose	0	0	24	24	1.6
Redhead	0	0	23	23	1.5
Lesser Scaup	0	0	22	22	1.5
Canada Goose	0	0	11	11	0.7
Hybrid Duck	0	9	0	9	0.6
Wood Duck	4	2	3	9	0.6
American Coot	0	0	7	7	0.5
Ring-necked Duck	0	0	6	6	0.4
Ruddy Duck	0	0	6	6	0.4
Common Goldeneye	0	0	5	5	0.3
Hooded Merganser	0	0	5	5	0.3
Bufflehead	0	0	4	4	0.3
Trumpeter Swan	0	0	3	3	0.2
Barrows Goldeneye	0	0	2	2	0.1
Total	261	191	1050	1502	100

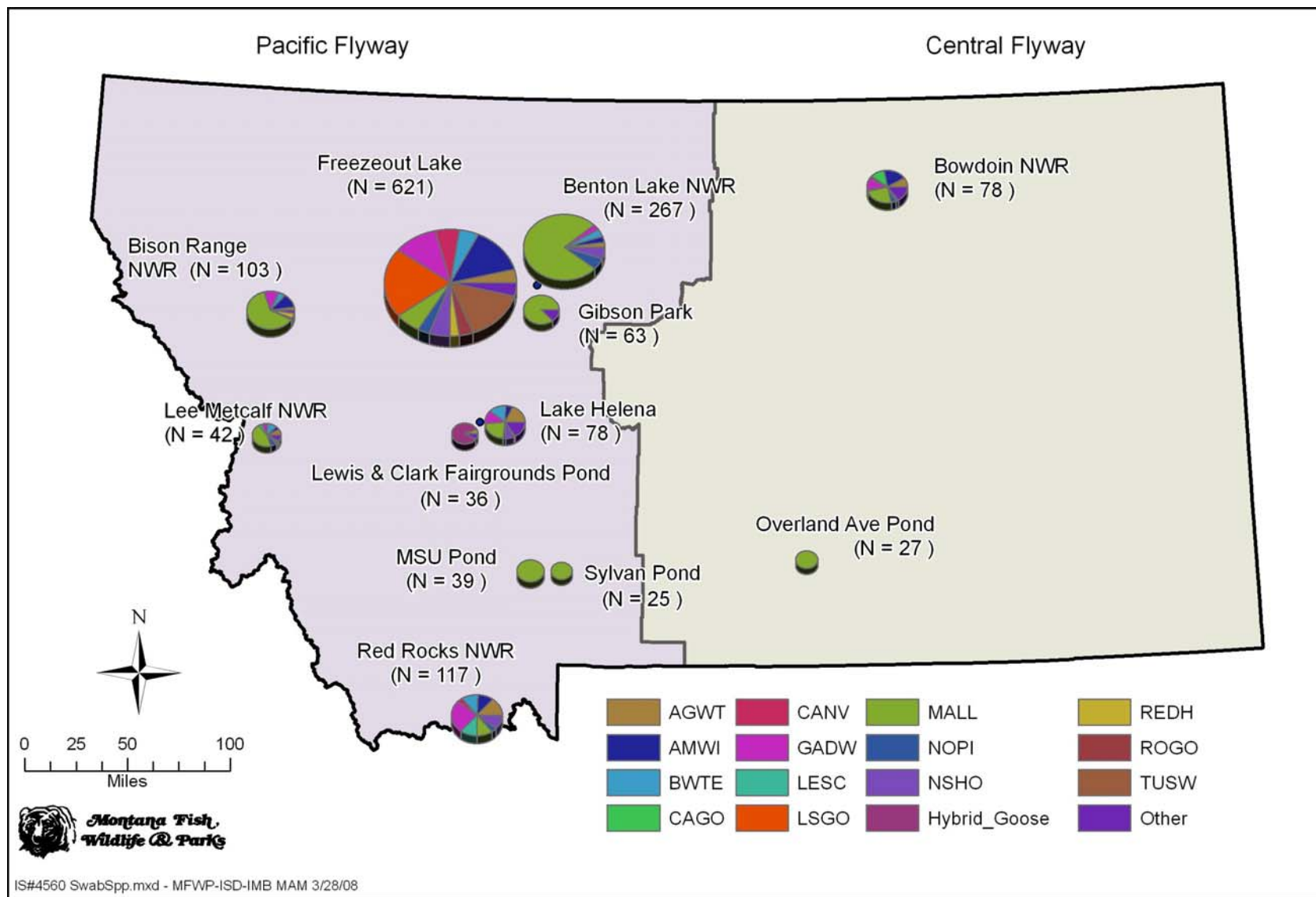
Age class was divided into hatch-year, after-hatch-year, and undetermined, and sex classification was divided into female, male, and undetermined. Slightly more than half of all birds sampled were classified as hatch-year (n=1076, 53.2%) while fewer were classified after-hatch-year birds (n=900, 44.5%). Age for 46 birds sampled (2.3%) was not determined. Within species, northern pintail, gadwall, northern shoveler, American wigeon, and blue-winged teal hatch-year birds were sampled in highest numbers (~70-75%) while lesser snow goose, mallard, and green-winged teal age classes were sampled quite evenly. Tundra swan and common goldeneye after-hatch-year birds were sampled in higher numbers (~70%) than hatch-year birds (Table 4).

Table 4. Number of the 2007 Montana AI cloacal-orpharyngeal samples according to species, age, and sex classes. The six samples from undetermined aged birds (2 male mallards, 3 male American wigeon, 1 female northern shoveler) were excluded.

Species	Number of hatch-year		Number of after-hatch-year		Number of undetermined sex		Total Number
	Male	Female	Male	Female	Hatch-year	After-hatch-year	
Mallard	134	122	152	127	1	0	536
American Wigeon	44	53	20	16	3	0	136
Gadwall	34	54	27	23	0	0	138
Lesser Snow Goose	0	0	0	0	37	78	115
Tundra Swan	0	1	10	7	25	50	93
Blue-winged Teal	28	34	5	11	0	1	79
Northern Shoveler	21	32	6	11	0	0	70
Green-winged Teal	17	23	15	12	0	0	67
Canvasback	6	19	14	9	0	0	48
Northern Pintail	17	13	7	10	0	0	47
Hybrid Goose	0	0	0	0	0	31	31
Ross's Goose	0	0	0	0	7	17	24
Redhead	6	5	8	4	0	0	23
Lesser Scaup	4	10	4	3	1	0	22
Canada Goose	2	0	2	3	3	1	11
Hybrid Duck	0	0	4	3	0	2	9
Wood Duck	3	2	4	0	0	0	9
American Coot	3	2	2	0	0	0	7
Ring-necked Duck	1	2	2	1	0	0	6
Ruddy Duck	0	0	4	2	0	0	6
Common Goldeneye	3	1	1	0	0	0	5
Hooded Merganser	0	2	0	3	0	0	5
Bufflehead	0	2	0	2	0	0	4
Trumpeter Swan	0	0	0	0	3	0	3
Barrows Goldeneye	0	0	1	1	0	0	2
Total	323	377	288	248	80	180	1496

Most tundra swan, lesser snow goose, Ross's goose, and northern pintail samples were collected in northwestern Montana at Freezeout Lake (Figure 3). Mallards were sampled at all sites across the state and were distributed throughout western and central Montana. The majority of the remaining species sampled were spread across the western and central parts of the state. Hunter-harvested birds provided the greatest species diversity for sampling, whereas urban trapping allowed for little diversity given nearly all birds available for trapping at ponds were mallards and hybrid geese and ducks.

Figure 3. Spatial distribution of the 2007 Montana AI cloacal and oropharyngeal sampling according to species. The “Other” category combines all species from which ≤ 11 samples were collected (n= 67, Table 3). National Wildlife Refuge is referred to as “NWR”. National Wildlife Refuge is referred to as “NWR”.



The collection of samples from primary species began with northern pintails on 9/5 and peaked 9/29; the majority of samples were collected during hunter-harvest sampling. Tundra swan sampling began 10/21 and peaked 10/27, lesser snow goose sampling also began 10/21 and peaked 11/13, while Ross's goose sampling began 10/27 and peaked 11/13; samples for all three species were collected from hunter-harvested birds. Sampling of the primary species ended in mid-November (Figure 4). Sampling of secondary species began with mallards on 8/16 and peaked on 9/29 during the opening day of waterfowl hunting. Consistent mallard sampling was highest during refuge banding operations and extended throughout the sampling season, ending in early December. Gadwall, American wigeon, and northern shoveler sampling began 9/5, 9/13, and 9/22, respectively, and peaked on 9/29 as well. Gadwall sampling ended in late October while American wigeon and northern shoveler sampling ended during mid-November (Figure 5). Additional species sampled in numbers >30 included blue-winged teal (began and peaked 9/29, ended 10/28), green-winged teal (began 9/12, peaked 9/29, ended 11/13), and canvasback (began 9/23, peaked 9/29, ended 10/28). Sentinel species (hybrid geese and ducks) were sampled at urban ponds from late October through early December.

Effort for cloacal and oropharyngeal sampling was divided evenly between MFWP and WS. MFWP spread sampling temporally throughout fall between urban trapping, refuge banding and hunter-harvest sampling, beginning 8/16 and ending 12/5. WS focused mostly on hunter-harvest sampling with 2 days each on refuge banding and urban trapping, beginning 9/5 and ending 12/1. Sampling for both agencies peaked 9/29, the opening day of waterfowl hunting in Montana (Figure 6).

Figure 4. Temporal sampling distribution of primary species for the 2007 Montana AI Surveillance Project.

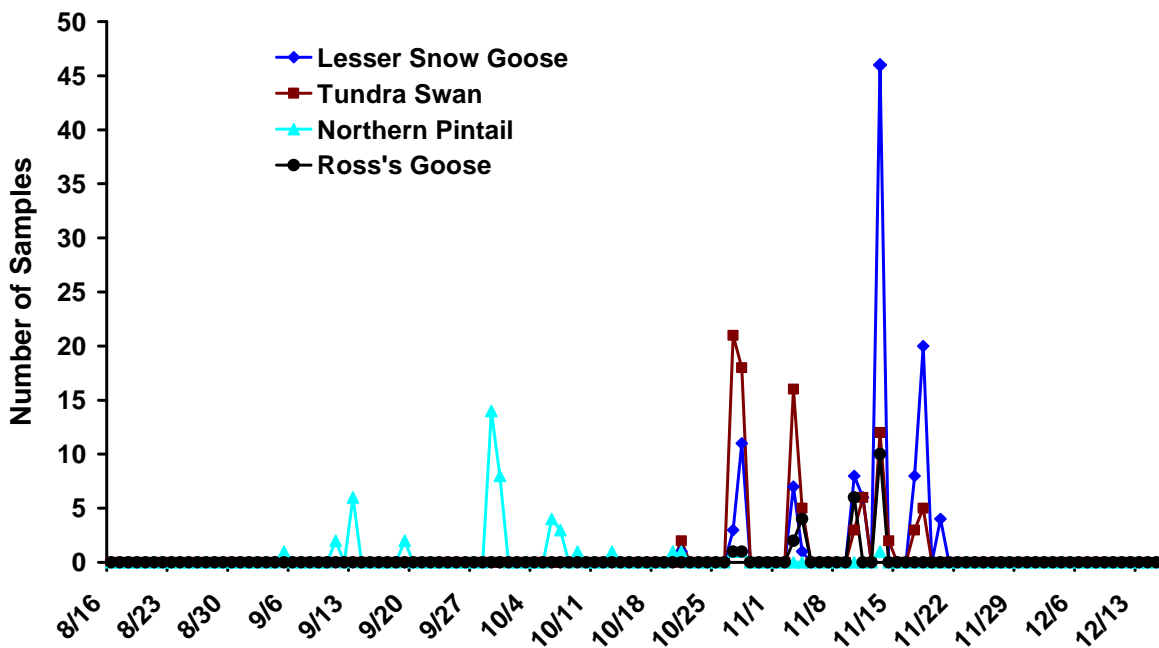


Figure 5. Temporal sampling distribution of secondary species for the 2007 Montana AI Surveillance Project.

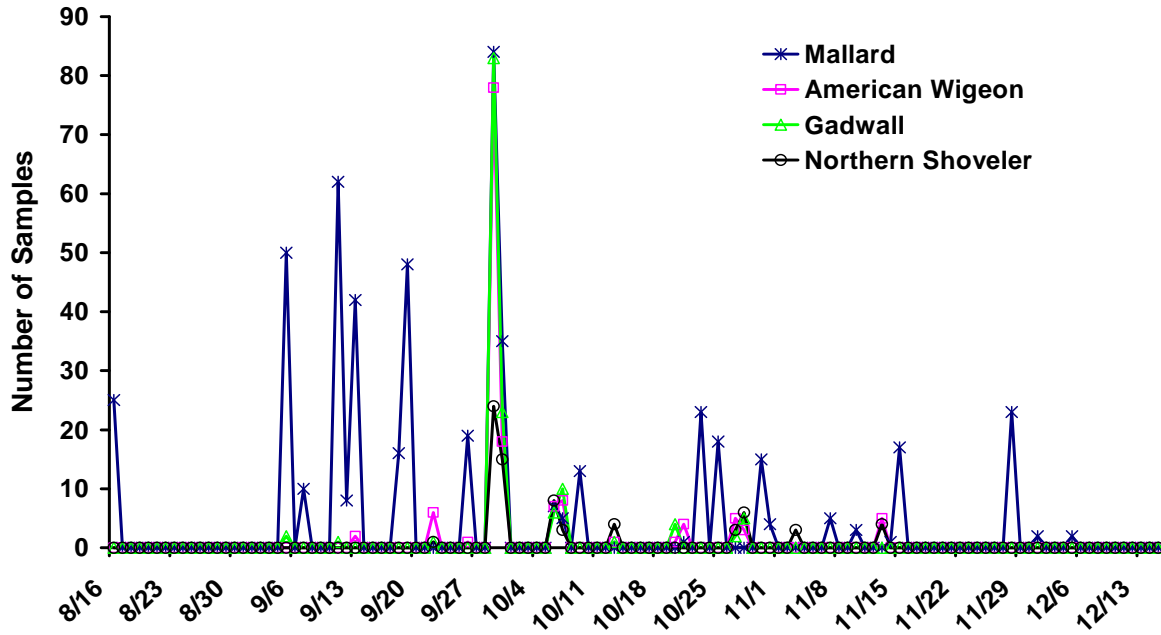
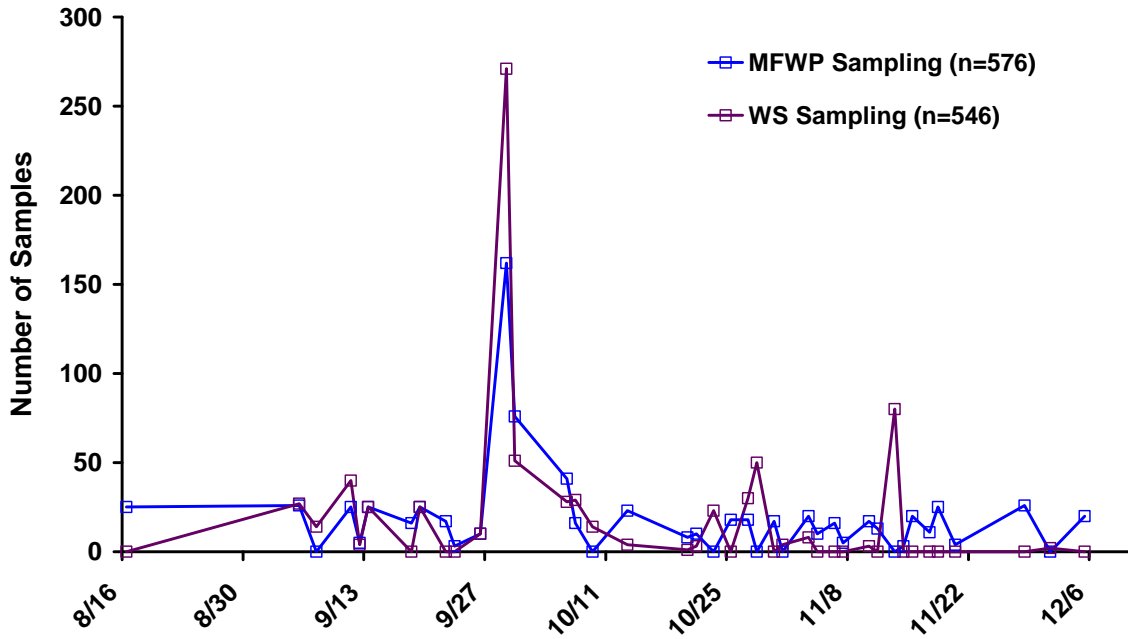


Figure 6. Temporal distribution of Montana 2007 cloacal and oropharyngeal sampling according to agency.



Environmental Sampling

Field

WS was responsible for the collection of 700 environmental samples across the state from August through December. Environmental sampling ran concurrently with cloacal and oropharyngeal sampling and was spatially and temporally distributed throughout the sampling period. According to the 2007 AI sampling criteria, batches of 20-30 individual specimens per sampling session were to be collected at pond levees, boat docks, dikes or dams, and shorelines. Samples taken from the same location were to be collected at least three weeks apart to reduce the likelihood of duplicating specimens from the same birds (USDA-APHIS-Wildlife Services et al. 2006). Environmental sampling sites included Castle Reservoir, Freezeout Lake, Helena Regulating Reservoir, Legion Pond near Billings, Medicine Lake National Wildlife Refuge, War Horse Reservoir, and Wild Horse Reservoir. Fresh feces (<24 hours old) were collected with swabs and placed in cryovials containing bovine albumin diluent to preserve the samples and virus particles, if present. As with the cloacal and oropharyngeal samples, pre-printed barcodes were placed on the vials and corresponding USDA lab submission forms. The date, collector, county and site, location in WGS 84 decimal degrees, and the three most abundant species at each site were recorded, as well as a sample batch referral number, submitter, and number of samples in each shipment. Samples and related lab submission forms were shipped overnight to the WS National Wildlife Research Center in Fort Collins, CO, in Styrofoam[®]-lined boxes with cold packs within 48 hours of sample collection.

Lab

Up to five individual environmental samples were combined to form sample pools that were treated with an inhibitex compound to remove natural inhibitors in the fecal samples. Pooled samples were tested using rRT-PCR following the same protocols as described for the cloacal-oropharyngeal samples to detect AI viruses. If positive, pools were tested again with rRT-PCR for H5 and H7. Presumptive and suspect H5 and H7 positive pools were then sent within 48 hours to NVSL for confirmatory testing that followed cloacal-oropharyngeal sample testing protocols.

Sampling Effort

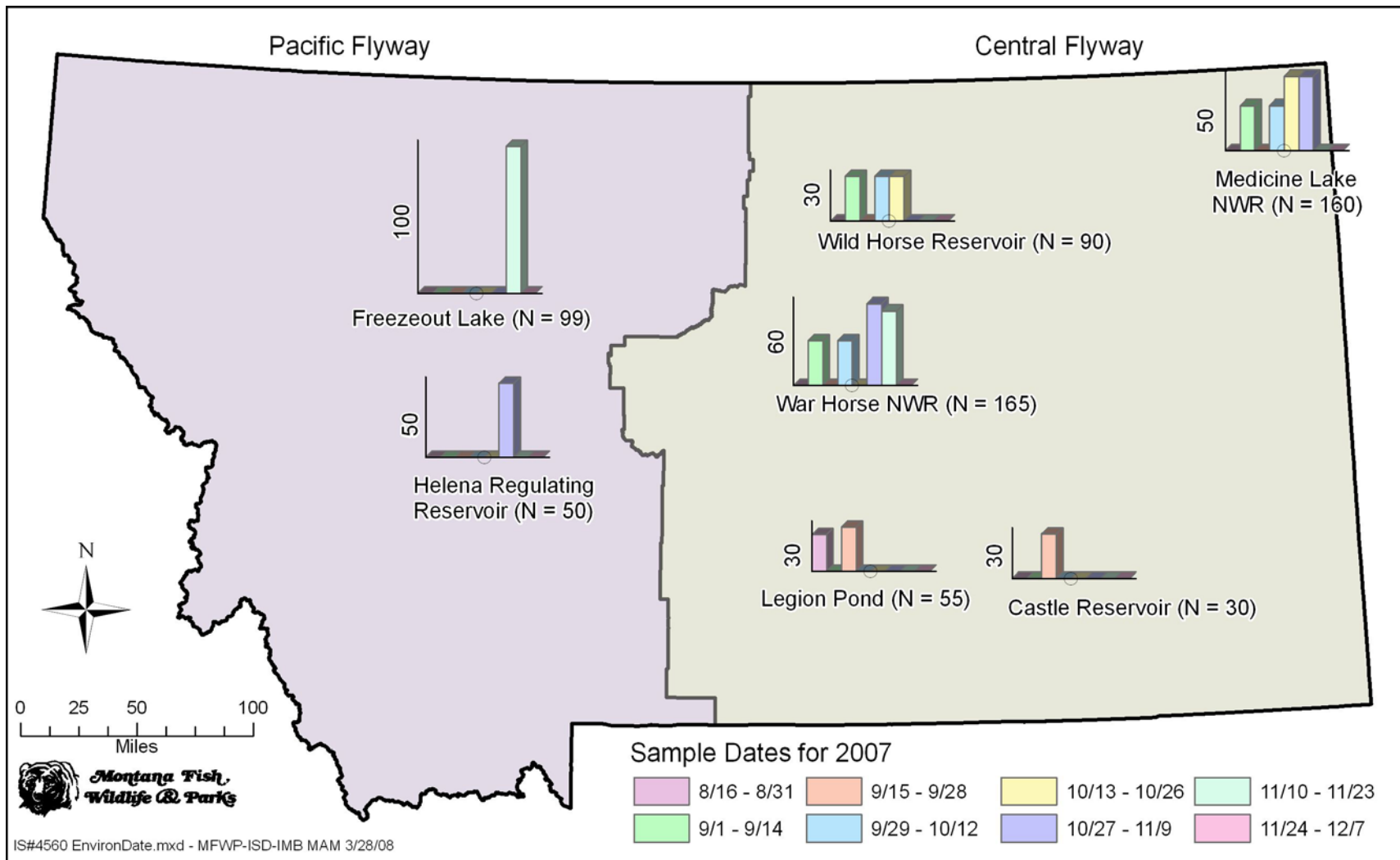
WS collected 649 environmental samples on 17 sample days from 8/8 through 11/19 at 7 sites statewide. The mean number of sample days/total number of sites was 2.4 and the mean number of samples/sample day was 38.2. The largest numbers of samples were collected at War Horse Reservoir (n=165) and Medicine Lake (n=160), which comprised half of all samples collected (Table 5).

Table 5. Number of sample days, and number and percentage of the 2007 Montana AI environmental samples collected according to site.

Site	Sample days	Total number of samples	Percentage of total samples
War Horse Reservoir	4	165	25.4
Medicine Lake	4	160	24.6
Freezeout Lake	2	99	15.3
Wild Horse Reservoir	3	90	13.9
Legion Pond	2	55	8.5
Helena Regulated Reservoir	1	50	7.7
Castle Reservoir	1	30	4.6
Total	17	649	100

Consistent environmental sampling across the state began in early September and peaked in mid-November at Freezeout Lake. Most sampling was conducted in the Central Flyway and was distributed evenly through the sampling period; sampling in the Pacific Flyway was conducted at two sites during late October and mid-November. Environmental sampling was for the most part conducted in areas not used for the other sampling methods (Figure 7).

Figure 7. Temporal distribution of the 2007 Montana AI environmental sampling. Scale bar numbers are the maximum number of samples collected during a two-week sample period. National Wildlife Refuge is referred to as “NWR”. National Wildlife Refuge is referred to as “NWR”.



Mortality/Morbidity Sampling

The 2007 Montana Sampling Plan Supplement specified the collection of ≤ 200 opportunistic mortality/morbidity samples during the 2007 sampling period. In 2006, MFWP established a toll-free number and a web-based reporting system on the MFWP website through which the public could report dead or sick birds. The MFWP AI Coordinator and Wildlife Lab Supervisor determined which of the reports made by the public were investigated according to the 2007 AI sampling criteria. These criteria included consideration of the reported species as a potential concern for the presence of HP-H5N1 and the circumstances under which the dead or sick birds were found. Morbid birds were euthanized in accordance with the Guidelines for Euthanasia of Non-domestic Animals (AAZV 2006) and entire carcasses were shipped within 24 hours for necropsy and disease testing at NWHC in Madison, WI. Bird carcasses suitable for disease testing found within 24 hours of death were also shipped to NWHC. Some samples were sent to the MDoL lab in Bozeman to expedite the reporting of cause of death for mortality events. If shipment within 48 hours of death was not possible, carcasses were frozen and shipped as soon as possible. The NWHC lab submission form contained the name of the submitter, date of carcass collection, location data recorded in WGS 84 decimal degrees, whether the bird was euthanized or found dead, and environmental data where the bird was found. The species, age, sex, condition of the bird, and clinical signs of disease were also recorded. Mortality event onset and end date, known and estimated number of dead birds, potential at-risk species, and bird population movements were recorded.

Lab

NWHC tested tracheal and cloacal swab samples and tissues by direct extraction. Testing procedures followed those described for cloacal-orpharyngeal sample testing and samples that tested positive for either H5 or H7 were sent to NVSL for confirmation. Samples that tested matrix RT-PCR positive but not H5 or H7 positive were submitted for virus isolation as time and space allowed in the NWHC laboratory.

Sampling Effort

A total of 59 mortality/morbidity samples were collected by MFWP and USFWS from 24 species that included birds from 27 mortality events reported statewide (Table 6). The 32 calls received on the MFWP toll-free reporting system and two website reports of dead and dying birds yielded five mortality/morbidity sampling events. Multiple-bird mortality events at Priest, Georgetown, and Smith Lakes, Rattlesnake Reservoir, and Medicine Lake and Bowdoin, as well as single-bird mortalities across the state were investigated. Fifty-one carcasses were submitted to NWHC and the remaining eight carcasses were submitted to the MDoL lab for AI testing. Of the 27 birds categorized by age and sex, 17 were classified as hatch-year birds (5 females, 11 males, 1 undetermined), eight were classified as after-hatch-year birds (3 females, 5 males), and two were classified as undetermined age (1 female, 1 male).

Table 6. 2007 Montana AI mortality/morbidity samples submitted to NHWC and MDoL labs according to species.

Species	Number of samples
American Coot	12
Bohemian Waxwing	6
Lesser Snow Goose	6
American White Pelican	5
Pine Siskin	4
Mallard	3
American Crow	2
House Sparrow	2
Northern Shoveler	2
Ring-billed Gull	2
Western Grebe	2
American Green-winged Teal	1
American Robin	1
California Gull	1
Common Grackle	1
Eastern Kingbird	1
Gadwall	1
Great Blue Heron	1
Gray Catbird	1
Redhead	1
Ring-necked Duck	1
Sora	1
Trumpeter Swan	1
Tundra Swan	1
Total	59

Mortality/Morbidity Transects

Prospective mortality/morbidity surveillance was added as an AI detection method by the USFWS in 2007. MFWP AI personnel conducted six weekly mortality transects of approximate equal length to systematically survey species of concern throughout the state of Montana for morbidity and mortality (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). Species identified as sensitive to HPAI infection that resulted in clinical disease and death were targeted for surveillance from the time they arrived in fall during migration until freeze-up. Priority species included tundra and trumpeter swans, American wigeon, canvasback, lesser scaup, northern shoveler, redhead, ring-necked duck, and wood duck, as well as shorebirds, grebes, terns and gulls (Becker 1966, Brown et al. 2006, Brown et al. 2008). Reconnaissance based on historical MFWP avian inventory data was conducted on 27 lakes and wetlands to find sites containing a minimum of ten target species for prospective mortality/morbidity surveillance. Of those, ten lakes and wetlands throughout the Pacific and Central Flyways were identified as suitable and used during 2007 surveillance based on location, water conditions, access, and target species abundance. Surveillance was conducted within 5-9

days of the previous survey and continually evaluated based on the presence of priority species. Surveys were performed consistently at six sites across the state; alternate locations were substituted for sites when target species abundance declined in October and November due to migration. Transects were terminated when total target species numbered ≤ 200 , a site was inaccessible due to winter conditions, or the lake or wetland froze over.

Mortality/morbidity transects contoured within ten feet of the shoreline to detect morbidity and mortality events either by canoeing or walking. Entire shorelines were surveyed on small bodies of water, while transects were established along portions of shoreline where the largest concentration of target species was found on large bodies of water. To record target species presence and an index of abundance, censuses were conducted with spotting scopes and high-powered binoculars. Censuses were consistently conducted from a single point on each transect that allowed maximum visibility to the observer. To avoid double counting during the performance of individual transects, only numbers of each species counted upon initial sighting were recorded to yield a minimum number, and only counts of additional target species not seen during the initial census were added during the transect. Because it is likely bird populations were resampled across consecutive surveys, the census data are reported as “bird observations”. Census locations and transect routes were recorded using Vista GPS units. The date, observer, site, location in WGS 84 decimal degrees, as well as the transect, start and end time, and total survey time were recorded on MFWP datasheets. Environmental data were also recorded, including temperature, cloud cover, precipitation, and wind speed, and whether the transect was walked or canoed and percentage of the transect completed. Bird census data for primary species (swans and ducks) included species, sex, and age when possible. Species, sex, and approximate ages were identified via plumage (Carney 1992). A category of unknown was assigned when it was not possible to distinguish between adult female and juvenile ducks in basic plumage and adults and juveniles after first molt in early fall. Counts of secondary species of concern (shorebirds, grebes, gulls, and terns) were also recorded. All symptomatic or dead birds of suitable quality were collected and tested for AI by submission of intact carcasses to NWHC following the protocols described above, and species and numbers of dead and morbid birds found while performing transects were recorded.

Sampling Effort

Reconnaissance for ponds and lakes to be used in mortality/morbidity transects began 7/5 and was conducted on 28 lakes and wetlands across the state (Figure 1). Ten sites were chosen for mortality/morbidity transects based on the presence of target species. A total of 103 transects were conducted between 7/18 and 11/21 throughout the state of Montana. Transect routes ranged from 2 to 9 km in length for a total of 41 km and averaged 4.1 (± 1.47) km. Completed surveys ranged from 54 to 360 minutes and averaged 140 (± 10.48) minutes for a total of 217 hours (Table 7). A total of 45,195 bird observations were recorded upon initial sighting of target species during the transects, of which nearly half were ducks, geese, and swans. Nearly one fifth of all bird observed were American coots on Georgetown Lake and the remaining third were gulls, terns, shorebirds, grebes, cranes, and one loon (Table 8). Dead and sick birds found on transects totaled 1584 and 329, respectively, the majority of which were American coots at Georgetown Lake (n=1546 dead, n= 328 sick). Eleven carcasses suitable for testing from Georgetown Lake and three from Eyraud Lakes were sent to NWHC for AI testing and to determine cause of death.

Table 7. 2007 Montana AI mortality/morbidity transect start and end dates, length and average survey times for complete surveys.

Transect	Date		Transect length (km)	Average survey time (min)	Number of surveys
	start	end			
Fox Lake	7/18	11/8	2	200	17
Brown's Lake	7/20	10/24	9	165	14
Lima Reservoir	7/25	10/5	2	140	11
Yellow Water Reservoir	8/1	11/19	2	120	17
Georgetown Lake	8/2	11/19	4	95	17
Eyraud Lakes	8/6	11/20	5	65	15
¹ Clark's Canyon Reservoir	10/17	10/25	2	185	2
² Warm Springs Ponds	10/31	11/20	6	225	4
³ Canyon Ferry Pond 2	10/31	11/21	6	130	4
⁴ Deadmans Basin Reservoir	11/16	11/20	3	70	2
Total	7/18	11/21	41	140	103

¹Clark's Canyon Reservoir replaced Lima Reservoir.

²Warm Springs Ponds replaced Brown's Lake.

³Canyon Ferry Pond 2 replaced Clark's Canyon Reservoir.

⁴Deadmans Basin Reservoir replaced Fox Lake.

Table 8. Montana 2007 mortality/morbidity transect bird observations according to family.

Family	Number counted (%)
Anatidae (ducks, geese, swans)	20,962 (46.4)
Rallidae (coots)	8,842 (19.6)
Laridae (gulls, terns)	7,523 (16.6)
Scolopacidae (sandpipers, phalaropes)*	4,227 (9.4)
Podicipedidae (grebes)	2,922 (6.5)
Charadriidae (plovers, killdeer)	603 (1.3)
Recurvirostridae (avocets)	101 (0.2)
Gruidae (cranes)	14 (0.0)
Gaviidae (loons)	1 (0.0)
Total	45,195 (100)

*Includes curlews, dowitchers, godwits, sanderlings, willets, yellowlegs.

Data Management, Reporting of Results, Statistics

MFWP and WS AI personnel entered cloacal and oropharyngeal data into a USDA national web-based database system. USDA reported cloacal-oropharyngeal sample results through the USDA web-based database, which included H5, H7, and N1 screening results, as well as LPAI subtype and pathogenicity. All 2007 cloacal and oropharyngeal data and results were then uploaded to MFWP's existing AI database. The NWHC and MDoL labs reported mortality/morbidity results directly to MFWP. Reported results contained the outcome of AI, additional disease testing, and cause of death. MFWP created a separate database for the mortality/morbidity transect data while all AI mortality/morbidity data and results for carcasses sent to the NWHC and MDoL labs were entered into the existing MFWP AI database. USDA personnel entered Montana environmental data into a separate USDA national database where pooled matrix sampling results were reported according to location. USDA reported results for pools that tested positive for H5 and H7 without the location. Confidence intervals were calculated for the proportion of

matrix positive cloacal-orpharyngeal swab samples according to species (R Core Development Team, 2006). Using the Agresti-Coull interval, the assumptions were 1) sampling was random or at least representative of the entire population, 2) LPAI rates were the same temporally, spatially and across trapping methods, and 3) there was no measurement error. Confidence intervals for matrix positive cloacal-orpharyngeal swab samples according to sex and age classes were not calculated due to the large differences in the proportion of matrix positives within each sex and age class according to species and the result of pooling those differences.

RESULTS

While AI virus was found in samples, HP-H5N1 was not detected in Montana during the 2007 surveillance. Since the AI surveillance did not focus on the detection of LPAI, samples that tested matrix positive and H5 and H7 negative were not tested with VI to determine AI subtype. It is therefore not possible to report specific low pathogenic subtypes for the matrix positive samples found during the 2007 Montana surveillance.

Cloacal-orpharyngeal Samples

Matrix Results

Of the 1502 cloacal-orpharyngeal samples submitted for AI testing, 159 (11%) samples tested positive on the AI matrix. The hunter-harvest method yielded the highest percentage of samples for testing (70%) and matrix positive samples (48%), yet yielded the lowest percentage of matrix positive samples within the method (7%). Urban trapping produced the fewest samples (13%) and lowest percentage of matrix positive samples (10%), and nearly the same matrix positive samples within the method (8%) as the hunter-harvest method. While refuge banding produced a low percentage of the total samples for AI testing (17%), the method yielded a high percentage of the total matrix positive samples (42%) and more than one quarter of the total samples within the method (26%: Table 9).

Table 9. 2007 Matrix positive cloacal-orpharyngeal numbers and percentage according to method.

Method	Total number of samples (%)	Total number of matrix positives (%)	Percentage matrix positives of method total
Hunter-harvest	1050 (70)	76 (48)	7%
Refuge banding	261 (17)	67 (42)	26%
Urban trapping	191 (13)	16 (10)	8%
Total	1502 (100)	159 (100)	11%

Due to sample sizes, known sex and age classes across all sampled species and methods were pooled for temporal analysis, and August samples (n=25) were pooled with September samples. The proportion of hatch-year females and males that tested matrix positive during August and September was highest among all sex and age classes, and then decreased strongly in October (0.17 to 0.06 and 0.16 to 0.8, respectively). The proportions of matrix positive after-hatch-year females and males tested during August-September were each 0.13. While the proportion of

after-hatch-year males that tested matrix positive decreased to 0.03 in October, the proportion of matrix positive after-hatch-year females was the only sex and age class to increase in October to 0.15. All sex and age classes tested matrix negative in November, however, most samples sizes in the sex and age classes for November were small (Figure 8). The highest proportion of matrix positive samples within species was wood duck (0.22, n=9) and mallard (0.19, n=538), though the wood duck sample size was small. Among the primary species, the proportion of matrix positive samples was relatively low with 0.09 for northern pintail, 0.03 for lesser snow goose, 0.02 for tundra swan, and zero for Ross's goose (Table 10).

H5 and N1 Results

All 2007 positive results for H5 and N1 using rRT-PCR were from hatch-year bird samples using cloacal and oropharyngeal swabs and were collected during late October and early November. One female canvasback from Freezeout Lake, one male mallard from Benton Lake, and one female mallard from Lake Helena tested H5 positive and N1 negative using rRT-PCR. One male mallard from Benton Lake tested positive for H5 and N1 using RRT-PCR and VI, and the H5N1 virus was classified as low pathogenic using target amino acid sequence analysis. H7 was not detected in the 2007 Montana samples.

Figure 8. Proportion of Montana 2007 cloacal-oro-pharyngeal swab matrix positives according to known sex and age classes.

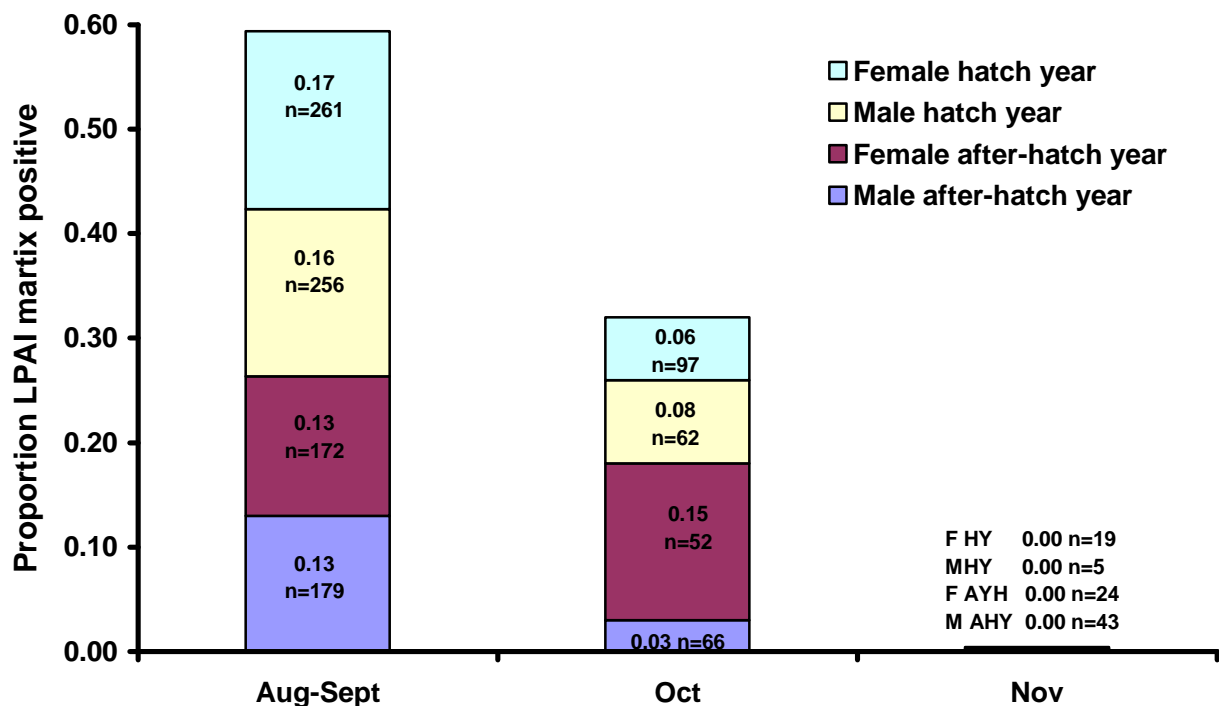


Table 10. Proportion of Montana 2007 cloacal-oro-pharyngeal swab matrix positive samples according to species using the Agresti-Coull interval. X= number of matrix positive samples within species, N= number of birds within species sampled, Mean= proportion of matrix positive samples within species, Lower CI= lower Confidence Interval, Upper CI= upper Confidence Interval.

Species (n=25)	X	N	Mean	Lower CI	Upper CI
Wood Duck	2	9	0.22	0.05	0.56
Mallard	104	538	0.19	0.16	0.23
Green-winged Teal	9	67	0.13	0.07	0.24
Blue-winged Teal	9	79	0.11	0.06	0.20
Hybrid Duck	1	9	0.11	0.00	0.46
Northern Shoveler	7	71	0.10	0.05	0.19
Canada Goose	1	11	0.09	0.00	0.40
Northern Pintail	4	47	0.09	0.03	0.20
American Wigeon	8	139	0.06	0.03	0.11
Lesser Scaup	1	22	0.05	0.00	0.24
Gadwall	6	138	0.04	0.02	0.09
Lesser Snow Goose	4	115	0.03	0.01	0.09
Tundra Swan	2	93	0.02	0.00	0.08
Canvasback	1	48	0.02	0.00	0.12
Hybrid Goose	0	31	0.00	0.00	0.13
Redhead	0	23	0.00	0.00	0.17
Ring-necked Duck	0	6	0.00	0.00	0.44
Ross's Goose	0	24	0.00	0.00	0.16
Ruddy Duck	0	6	0.00	0.00	0.44
Trumpeter Swan	0	3	0.00	0.00	0.62
Hooded Merganser	0	5	0.00	0.00	0.49
American Coot	0	7	0.00	0.00	0.40
Barrow's Goldeneye	0	2	0.00	0.00	0.71
Bufflehead	0	4	0.00	0.00	0.55
Common Goldeneye	0	5	0.00	0.00	0.49

Environmental Samples

The 649 environmental samples collected produced 130 sample pools, of which six pools (4.6%) yielded positive results for AI virus. Three locations in the Central Flyway (Wild Horse Reservoir, Castle Reservoir, Medicine Lake) and one location in the Pacific Flyway (Freezeout Lake) produced matrix positive results, the highest number of which were from samples collected during mid-September (n=4; Table 11).

Table 11. Number and date of environmental sample pools that tested positive for the AI matrix during the 2007 Montana AI surveillance.

Site	Number of pools	Date sampled
Wild Horse Reservoir	1	9/12
Castle Reservoir	3	9/19
Medicine Lake	1	10/22
Freezeout Lake	1	11/13
Total	6	-----

Mortality/Morbidity Samples

The 59 mortality/morbidity samples tested for AI virus produced six presumptive positives based on virus isolation in tissues, all of which were tested by NWHC. Samples from two hatch-year males, one gadwall collected on 9/12 from the Kalispell area and one western grebe collected on 11/14 from Georgetown Lake, produced positive results for the AI matrix. Four American coots collected on 10/4 from Smith Lake also produced positive results for the AI matrix. All six birds were tested with cloacal and tracheal swabs using rRT-PCR and all results were PCR-negative, while VI tissues sampling from the same birds yielded positive results for AI virus. Since no positive results for H5 or H7 were detected, NWHC did not test for N1, LPAI subtype, or pathogenicity. Cause of death for mortality events will be reported by MFWP.

DISCUSSION

AI virus in low pathogenic form was detected in Montana samples as expected, while HP-H5N1 was not found during the 2007 surveillance in Montana or elsewhere in North America. The male hatch-year mallard that tested H5N1 positive via rRT-PCR and VI was classified as low pathogenic using target amino acid sequence analysis. This was the only bird determined to have H5 and N1 linked in the same strain during the two years of surveillance in Montana. However, the low pathogenic classification means the HP-H5N1 Asian strain of concern was not detected.

Within sampling methods, hunter-harvest sampling produced the most samples (70%) and lowest percentage of matrix positive samples (7%) while refuge banding yielded the most matrix positive samples (26%). Timing of refuge banding versus hunter-harvest and urban trapping sampling may partially explain this difference. Several studies have shown that AI is more prevalent in early fall and decreases as fall migration proceeds (Stallknecht 2003, Gilbert et al. 2006). Changes in LPAI concentration may be due to a combination of premigration density of waterfowl with the high recruitment rate of immunologically naïve juveniles in early fall, while subsequent declines in LPAI may be a result of increased flock immunity and progressive

dispersal of bird populations (Stallknecht 2003, Gilbert et al. 2006). The use of different trapping methods may also be a factor in differing low pathogenic AI results.

All six of the Montana mortality/morbidity LPAI positive samples were rRT-PCR negative via cloacal and tracheal swabs but VI positive using tissue sampling. Differences in results using rRT-PCR and VI were also reported in Alaska (Runstadler et al. 2007), and lower diagnostic sensitivity was found in recent H7 testing using rRT-PCR versus VI in California (Xing et al. 2008). One concern was the use of tests designed for and tested on domestic poultry but used on wild birds (Xing et al. 2008). Differences in the detection of AI virus between the assays may also be explained in part by what they detect; PCR detects RNA and VI detects only live virus. Factors that might adversely affect the sensitivity of PCR assays include substances that might inhibit the detection of RNA in the sample, inefficient RNA extraction, the potential of RNA to rapidly degrade before testing (Spackman et al. 2002, Runstadler et al. 2007), and differences between primer and probe sequences (Runstadler et al. 2007, Xing et al. 2008). Since VI can detect only live virus, negative results could be due to dead virus in the sample rather than the absence of the virus (Spackman et al. 2002) or decreased sensitivity due to inherent difficulties of sample storage, handling, and growth in embryonating eggs (Runstadler et al. 2007). VI is currently the gold standard assay to test for these viruses and the results are therefore considered definitive.

Success of wild live and hunter-harvested bird sampling, as well as mortality/morbidity sampling, depended on the availability of the species and numbers of birds during migration. Of the primary target species, lower numbers of northern pintails were sampled during 2007 (n=47) than in 2006 (n=219) primarily due to differences in numbers available for banding at Benton Lake. Lesser snow goose sampling also decreased between years (2006: n=151, 2007: n=115) while tundra swan sampling increased (2006: n=52, 2007: n=93). The timing of migration can be affected by many factors, including climate and weather patterns (Blokpoel and Richardson 1978, Nichols et al. 1983, Harmata et al. 2000), age of the migrants (Hepp and Hines 1991), population size (Nichols et al. 1983), and bird body mass, especially in hatch-year birds (Owen and Black 1989). It was important to obtain high numbers of hatch-year bird samples because that age class likely contained the highest prevalence of AI viruses during their first fall migration (Olsen et al. 2006); this was accomplished during the 2007 Montana AI surveillance. Mallard was the most abundant and available species for sampling in Montana and was therefore sampled strongly during refuge banding and urban trapping. However, to maximize sampling of other target species, the 2007 Mallard sampling was reduced to half of the previous year (2006: n=1072, 53% of total samples, 2007: n=536, 36% of total samples). While urban trapping provided the greatest flexibility temporally, as sampling could be conducted according to schedule rather than opportunistically, it afforded the least diversity of species among the methods (n=4). Conversely, hunter-harvest sampling was difficult to allocate temporally while it provided the most species diversity (n=21); 53% of the total hunter-harvest samples were collected during the first weekend of the waterfowl hunting season when the majority of hunting took place, after which sampling tapered quickly. Refuge banding, which provided one-sixth of all cloacal-orpharyngeal samples among six species, was concentrated during the month of September and conducted mostly at Benton Lake (15% of total cloacal-orpharyngeal samples). To spread sample collection temporally during the 2007 surveillance, additional emphasis was placed on wild sentinel birds at urban ponds and sampling during refuge banding, while hunter-harvest sampling was used to target a broad range of specific species.

The national 2008 AI surveillance is underway. Mortality/morbidity transects and environmental sampling began in July and sampling of live birds in Montana began in August. Opportunistic mortality/morbidity samples are collected throughout the year.

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